Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

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Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope
Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - *Panel A*
Z-Stack =86 Slices (8.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 1058 X 979
Image Size (Scaled) = 151.14 µm X 139.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 44.75 mm, Y: 44.46 mm
Stage Position = X: 44.75 mm, Y: 44.46 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - *Panel B*
Z-Stack = 40 Slices (3.9 µm) - *Gallery Subset was Used*

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm

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Image size (Pixels) = 322 X 301
Image Size (Scaled) = 46.00 µm X 43.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 44.75 mm, Y: 44.46 mm
Stage Position = X: 44.75 mm, Y: 44.46 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -
Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.20 µm
Binning Mode = 2,2

488 -
Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI - *Acquired but not shown in Data Analysis*
Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x

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Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.
Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

3D Image Processing - *Panel A*

3D Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - *Panel B*

Clipping planes are introduced in Panel B to highlight the spatial organization of Rabbit anti-Human Chk1 Polyclonal, IgG and Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3) within the nuclear compartment by applying a triple-wedge cut through the X–Y, X–Z, and Y–Z planes, selectively removing portions of the volumetric reconstruction to improve visualization of intranuclear signal distribution and colocalization.
X-Y = Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -20%
Clip X = 0°
Clip Z = 0°
X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -8%
Clip Y = 45°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = 11%
Clip Y = -30°
Clip Z = 0°

Panel B shows a wedge-cut view displayed from the top with a slight backward rotation, highlighting the region of wedge removal and revealing the full depth of signal across the z-stack. This orientation provides an alternative view of wedge geometry and confirms intranuclear localization of Chk1, as indicated by colocalization with ATRIP.

Summary

Widefield structured illumination microscopy (SIM) demonstrates coordinated intranuclear organization between Chk1 and ATRIP following etoposide-induced DNA

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damage. Rabbit anti-Human Chk1 Polyclonal, IgG was visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody. In contrast, Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), was visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. Three-dimensional maximum-intensity and wedge-view orthogonal projections reveal overlapping yet structurally complex signal organization throughout the nucleus, with interspersed regions of apparent colocalization consistent with activation of the ATR-mediated replication stress response pathway.

Although widefield SIM improves lateral resolution and volumetric interpretation relative to conventional widefield microscopy, substantial signal convolution remains present within dense nuclear repair domains. Nevertheless, orthogonal X–Y, X–Z, and Y–Z reconstructions demonstrate coordinated spatial distribution between Chk1 and ATRIP, supporting their functional association within DNA damage signaling microenvironments.

Biologically, ATRIP serves as the obligate recruitment partner of ATR and localizes to RPA-coated single-stranded DNA generated during replication stress and DNA end resection. Activation of the ATR-ATRIP complex results in phosphorylation and downstream activation of Chk1, a central checkpoint kinase that stabilizes the replication fork, induces S-phase arrest, and orchestrates DNA repair. The overlapping intranuclear architecture observed here is therefore consistent with coordinated recruitment of ATRIP and Chk1 to replication-associated damage domains following etoposide-induced genotoxic stress.

Image Correction

NONE

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

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